

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:	)
F. Bergmann, et al.	) EXAMINER: David C. Thomas
SERIAL NO.: 10/540,406	) ART UNIT: 1637
FILED: June 24, 2005	)
FOR: METHOD FOR BISULFITE TREATMENT	) Confirmation No. 8359
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DECLARATION OF THE INVENTOR  
IN RE: FINAL OFFICE ACTION DATED JULY 16, 2007

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

I, Frank Bergmann, am one of the inventors of the present application. I can easily read and understand English. I was asked to make this declaration in order to establish certain scientific facts. All of the following statements made of my own knowledge are true and all statements made on information and belief are believed to be true.

I obtained a Ph. D. degree in chemistry from the University of Konstanz, Germany in 1993. I have been a practicing research scientist in the field of nucleic acid chemistry for 14 years.

My co-workers and I have invented an improved method of bisulfite treatment of nucleic acids. Bisulfite treatment results in selective deamination of certain nitrogenous bases in the nucleic acids. This reaction is well-known in the art, however it usually takes place over a long time (16 to 18 hours). We have discovered a way to conduct the reaction in only 2 to 3 hours. We have found that the critical parameters that allow the deamination reaction to proceed quickly and efficiently are temperature and pH. In our experience, the optimal pH for this reaction is between 5.25 and 5.75 more precisely, around 5.5.

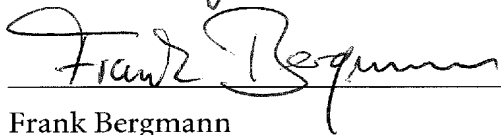
It is my understanding that a paper by Grunau et al. (Nucleic Acids Res. (2001) 29:e65, 1-7) teaches a similar yet different method of bisulfite treatment. Specifically, Grunau teaches adding 1200 uL of the bisulfite solution at pH 5.0 to 110 uL of denatured DNA, still in solution of 0.3M NaOH. Grunau does not specifically mention the pH of the final reaction solution.

I prepared the sodium bisulfite solutions as taught by Grunau (Solution I, 3.87-4.26 M at pH 5.0 and Solution II, 5.2-5.69 M at pH 5.0). I mixed 1200 uL of each of these bisulfite solutions with 110 uL of 0.3M NaOH. I observed the pH of the resulting solutions to be 5.05 and 5.02. This is significantly different from the pH range of 5.25-5.75 or the optimum of 5.5 that we found for the bisulfite reaction. This pH near 5.0 is the currently used value. Based on my experience, as well as the data disclosed in my application, pH near 5.0 at elevated temperatures will result in significant degradation of the nucleic acid. We discovered that only higher pH, such as for example 5.5, allows to increase the temperature and perform a quick, efficient and specific reaction with much less degradation of the nucleic acid.

I understand that willful false statements and the like are punishable by fine or imprisonment, or both and may jeopardize the validity of my application or any patent issuing on the application.

I declare under the penalty of perjury under the laws of the United States that the foregoing is true and correct.

Executed on Jan. 16, 2008 in Penzberg, Germany.

  
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Frank Bergmann